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New Concepts in Therapeutic Photomedicine: Photochemistry, Optical Targeting and the Therapeutic Window

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Advances in optics technology, synthetic photochemistry, and the science of photobiology make it possible to think beyond phototherapy and photochemotherapy which is dependent on direct photochemical alteration of metabolites or direct phototoxic insult to cells. This report discusses another gender of photomedicine therapy which includes *in vivo* photoactivation of medicines, photon-dependent drug delivery, and manipulation of host and exposure source to maximize therapeutic index. These therapeutic manipulations are made possible because the skin is highly overperfused and because nonionizing electromagnetic radiation that enters skin and blood has adequate photon energy to cause electronic excitation. Radiation of 320–800 nm is not very directly phototoxic, is absorbed by a variety of relatively nontoxic photolabile molecules and has an internal dosimetric depth profile. This radiation can therefore be used to activate, deactivate, bind, release or biotransform medications *in vivo* in skin or other organs. The photochemist, synthetic chemist and photobiologist can collaborate to significantly increase therapeutic possibilities.

Visible light can be used to prevent bilirubin encephalopathy in jaundiced newborns and to remove cutaneous hemangiomas. Ultraviolet radiation can be used to treat a variety of common and uncommon skin diseases and often changes disabling disease to a manageable problem. Yet it is likely that the potential for therapeutic use of ultraviolet and visible radiation is much greater than presently realized. Present forms of phototherapy depend on direct effects of photons on abnormal cells or on metabolites present in excessive amounts. The photon itself is the intended pharmacologic or therapeutic agent (see Table). The genesis of these treatments was the observation of therapeutic effects of sunlight and the mode of development has been mostly trial and error utilizing available exposure sources.

Direct *in vivo* photochemical cell injury (phototoxicity) is also the most likely mechanism for photo*chemo*therapy. By supplying an exogenous chromophore that is somewhat site-specific and leads to a specific photochemistry, this form of treatment potentially increases target selectivity or treatment efficacy. Photochemotherapy combines the ease and uniform distribution of systemic drug administration with selective spatial effects achieved by radiation. In the last decade this form of therapy has been successfully reduced to practice in a somewhat rational and systemic way utilizing modern lamp design, radiometry and careful dosimetry and has achieved limited success in the form of oral psoralen photochemotherapy. However, the concept of photochemotherapy is most likely more important than any present application.

Advances in optics technology and the science of photobiology make it possible to think beyond therapy which is dependent on direct photochemical alteration of metabolites or direct phototoxic insult to cells. This report discusses another generation of photomedicine therapy which includes *in vivo* photoactivation of medicines, photon-dependent drug delivery and creative manipulation of host and exposure source to maximize therapeutic index. We also summarize some of the photobiologic and biologic properties of skin and blood which make such treatments possible and examine some of the variables available for therapeutic manipulation.

Knowledge of the ways in which radiation affects biological matter ultimately permits these therapeutic manipulations. Specific biomolecules within cells can be altered in precise ways without the tissue being entered by chemicals, physically invaded or disrupted. For these considerations radiation can be imagined as energy in transit; electromagnetic radiation (EMR) is energy that can be transmitted through a vacuum. Energy can be emitted from an excited species (source) and subsequently invested in another molecule a few nanometers or hundreds of millions of miles away. The source may be an electrically excited gaseous arc; the absorbing molecule may be a chromophore in a living skin cell. EMR has the properties of both waves and particles. Many of the interactions of electromagnetic radiation with matter are best described by wave properties. Refraction, diffraction, polarization, and classical scattering are good examples. The wave character is best described as transverse waves of rapidly alternating electric and magnetic fields which are perpendicular to each and to the direction of propagation. Any specific EMR may be described by either the frequency (number of wave oscillations per second) or the wavelength (distance travelled per oscillation). Because all EMR is propagated at the same velocity in a vacuum, frequency and wavelength have a precise inverse relationship.

On the other hand, certain properties of EMR are best understood by considering discrete bundles or particles of finite energy (photons or quanta). The energy of a single photon is proportional to its frequency, and therefore is inversely proportional to wavelength. When a photon is absorbed, all of the photon's energy is transferred to the absorbing molecule and the photon no longer exists. Absorption is a precise phenomenon which depends upon the "allowed" electronic states and transitions within the absorber and how these match with the energy of the photon. Absorption must take place in order for photochemistry to occur (Grotthus-Draper law), because the photon must supply activation energy for these reactions.

For some finite period of time, the excess energy presented to a molecule by a photon results in an "excited" state of the molecule. The mode of this excitation depends upon the amount of energy invested (photon energy). At very high photon energies, e.g., x-rays, interaction may cause electrons to be ejected from the absorbing molecule, i.e., photo-ionization of the molecule. Infrared photons (longer wavelength, lower energy per photon) typically excite rotational and vibrational states resulting in changes most often recorded as heat. Absorption of visible and ultraviolet radiation leads to electronic excitations—changes in the orbital shells that the molecule's or atom's electrons occupy, actually creating an "electronic isomer" of the unexcited ground state molecule.

The excited molecule may dissipate its excess energy as heat or re-emit it as EMR (fluorescence or phosphorescence). The

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Abbreviations:
EMR: electromagnetic radiation
PADDS: photon-activated drug delivery systems

Therapy with optical radiation (UV, VIS, NIR)

Mechanism	Examples (Disease Treated)
Direct Effects (Phototherapy)	
Selective phototoxicity	Psoriasis (UVB)
Photochemistry of metabolites	Hyperbilirubinemia (blue light) Uremic Pruritus (UVB)
Photosensitized effect (photochemotherapy)	
Selective phototoxicity	Psoriasis, mycosis fungoides (PUVA) Cancer (hematoporphryin photoradiation)
Indirect	
Photon activation or inactivation of medicines	
Photolabile liposome carriers	

excited molecule may be structurally altered or cleaved, or it may react chemically with other molecules. In biological systems the photochemical alterations may eventually result in changes in cell function, mutation, death or other responses. Membranes, enzymes, DNA, RNA and other biomolecules may be altered and macromolecular synthesis temporarily decreased. Repair enzymes attempt to remove photoproducts from DNA. Photoproducts and mediators are released from cell membranes, lysosomes, and other organelles. Unlike either higher quantum energy (x-rays, gamma rays) or lower quantum energy (far infrared, microwaves), the optical portion (ultraviolet, visible, near infrared) of the spectrum can produce a large variety of highly specific biologic effects. This is because the quantum energy of UV and visible photons corresponds to the activation of specific molecules or chromophores, usually resulting in specific alterations of those absorbing centers. Thus, it is possible to "drive" a given photochemical reaction to the relative exclusion of others by supplying specific chromophores and choosing the right wavelengths. The specificity with which photochemical reactions can occur is similar to that for pharmacologic agents or enzymes, yet we are only beginning to utilize this potential.

If radiation induces sufficient cell injury, whole organs react. For example in the skin, after a latent period of several hours, the classic signs of inflammation appear. Rubor, tumor, calor, dolor last for hours to days and are followed by epidermal hyperproliferation and hyperpigmentation. These responses most often depend on the energy per photon (wavelength) and the number of photons absorbed. The number of photons present at each wavelength depends on the particular properties of the radiation source and the operating conditions. The internal spectral dosimetric profile depends on the optical properties of the tissue. The optical properties of skin and blood should allow the use of photons to release, activate or deactivate chemicals or medications at specific sites and depths within tissue. This can be achieved in a variety of ways, some examples of which are given below.

AMPLIFICATION OF PHOTON SIGNALS

Within cells or organisms photons may act as signals; their effects can be amplified in a variety of ways that magnify photobiologic responses. Naturally occurring systems of amplification initiated by photochemical reactions include gene repression or depression, enzyme activation or inactivation, control of hormones (release, binding, biotransformation), and control of membrane properties [1]. Photons may affect enzymes by a variety of mechanisms [2] including induction or enhancement of protein synthesis. Nonionizing EMR may create the substrate for enzymatic action or influence binding to the substrate. Photochemical alterations of enzymes may activate or deactivate them by a variety of different and sometimes precise mechanisms. While short wavelength ultraviolet radiation may simply denature the protein, longer wavelength ultraviolet or visible radiation may cause isomerization or specific photochemical changes in an enzyme which diminishes, enhances or abolishes its activity. Photochemical conformational changes may occur before or after the enzyme substrate complex has occurred. Wavelengths as long as 855 nm have been found to be effective in markedly enhancing enzyme reaction rates [3].

Photosensitizers can be used to enhance the selectivity of the effect of photons on enzymes, to alter the action spectrum of photoinhibition of certain enzymes, or to modify natural enzyme inhibitors. For instance, if proflavin is reduced by visible light in the presence of ascorbic acid, its inhibition of α-chymotrypsin is diminished by a factor of 10 [4]. In addition, enzymes can be chemically modified by the addition of chromophoric groups to make the enzyme itself photolabile. For example, chymotrypsin is inactivated 5 times more effectively by the *cis* form of p-azophenyldiphenylcarbamylchloride than by the *trans* form. Radiation with 320 nm and 420 nm changes the ratio of isomers in favor of *cis* and *trans*, respectively, and therefore degree of inactivation can be reversibly controlled by selection of waveband radiation [5]. Similar systems occur with acetylcholinesterase [6,7] and aldolase [8]. The enzyme α-chymotrypsin can be bound to the *cis* form of a modifier which eliminates its activity. When exposure to 313 nm radiation converts the modifier to the *trans* form, it falls off the protein completely, restoring its activity so that one photon could result in 10^5 molecules of the product of the enzyme reaction in 10 min [9].

PERCUTANEOUS RADIATION OF BLOOD

Considering metabolic need, the skin is highly overperfused. Under normal conditions, there is little relationship between the quantity of blood vessels and blood in skin and the metabolism and function of cutaneous cells. The mean blood flow to the skin is 20 to 30 times greater than necessary for physiological and metabolic demands of the skin cells and the maximum flow may be more than 100 times the minimum flow [10]. This is largely because the cutaneous vascular system is a regulator of total body temperature. The skin may also be a normal or abnormal reservoir for blood. Therefore, because skin blood flow primarily serves the organism as a whole, an unusually large portion of the blood is available near the body surface for exposure to nonionizing EMR.

Skin blood flow has been measured by a variety of techniques which include heat exchange kinetics, plethysmography, clearance of inert gases, laser dopler shift, skin conductance, dye studies and radioactive tracers [11–13]. A nude subject at rest at an environmental temperature of 35°C has an average cutaneous blood flow of 230 to 380 ml per m^2 of body surface per minute [14,15]. This can easily be greatly increased and flow up to 1000 ml per m^2 per minute has been reported [16]. Flow certainly varies from site to site, being greatest in digits, where a 600-fold temperature dependent flow rate increased capability has been reported [17,18], and in the face and perioral areas [19]. When flow is accelerated, the skin usually also contains more blood at any one time but these 2 parameters do not always run parallel to each other.

Cutaneous blood flow therefore accounts for about 10% of basal cardiac output; as much as 10% of the blood may be in the skin during any time period. This amount can be increased significantly by exercise or tolerable heat load. By changing environmental temperature from 28°C to 35°C, skin temperature rises only 2°C but blood flow increases 3-fold [16]. At rest at a mean cutaneous blood flow of about 250 ml/m^2/min or 500 ml/min for an adult, an equivalent of the entire blood volume may pass through the skin 2 or more times during the time it takes to achieve one minimal erythema dose (about 20 min) of sunlight. With the increased heat load typically present in artificial exposure chambers and prolonged exposures, as much as 2 to 10 times the cardiac output may pass through the skin

during phototherapy. Much of this blood is exposed to longwave ultraviolet, visible and near infrared radiation, more so to the longer wavelengths present due to the optics of skin.

OPTICS TARGETING AND THE THERAPEUTIC WINDOW

Radiation of 320–800 nm has properties which make this waveband potentially useful for the purpose of selective (therapeutic) *in vivo* photochemistry utilizing exogenously supplied chromophore:

1. This radiation is not directly very phototoxic: it carries sufficient quantum energy to initiate photochemical reactions, yet is relatively innocuous to exposed human tissues other than the eye.

Cells are most efficiently injured directly by radiation of wavelengths shorter than 320 nm. The photon-induced inflammatory response in skin results from a complex cascade of events which are poorly understood; the chromophores and molecular mechanisms are not known and both may vary with the wavelength (photon energy) of radiation, but DNA is probably a primary chromophore and "target" responsible for many responses to wavelengths shorter than 320 nm. The manifestation of skin phototoxicity which has received the most attention is the delayed vasodilation which accompanies inflammation. The effectiveness of various portions of the ultraviolet spectrum in causing erythema varies considerably. The exact shape of the 250–290 nm portion of the erythema effectiveness curve depends on the time of observation, degree of erythema used as endpoint and other variables. However, from 290 to 320 nm the sensitivity of skin as measured by the delayed erythema decreases by several orders of magnitude. Compared to 290–320 nm radiation, wavelengths from 320 to 380 are more than 1,000 times less effective at inducing inflammation in human skin and 10,000 times less effective at reducing the viability and function of human peripheral blood lumphocytes. Wavebands beyond 400 nm have even smaller direct phototoxic potential and in most cases massive exposure doses or extreme intensities would cause cutaneous changes related to heat long before biologically significant photochemistry would occur.

Perhaps this situation has occurred because we evolved under sunlight with maximum irradiance in the 320–800 nm spectral region. It would not do for a terrestrial animal to be extremely photosensitive to this spectral region, and traits such as porphyria with destructive endogenous photosensitizers in general have clearly been selected against. Whatever the history of man's general tolerance to this spectral region, the situation allows us to supply specific chromophoric molecules and "trigger" them with 320–800 nm radiation, expecting that within wide limits, we can cause responses primarily to the exogenously-provided photochemical pathway of interest.

2. This radiation has photon energy which can cause electronic excitation in many organic molecules and can therefore cause *selective* chemical reactions.

The minimum activation energy for the formation of breakage of very weak bonds in organic photochemical reactions may correspond to photon energies of 1 to 2 ev., i.e., red and near infrared radiation. Thus, wavelengths in the UV, visible and near infrared regions can potentially be used for photochemotherapy and selective *in vivo* photochemistry. Photochemical reactions differ from thermal reactions in several fundamental and important ways. Thermal activation of a reaction involves collisional excitation of vibrational states, whereas photochemical reactions always are initiated by direct electronic excitation of a single molecule by a single (or rarely, 2) photons. Since the population of excited vibrational levels is related to temperature, a colligative property, a single thermally-driven reaction cannot be enhanced by changing temperature without affecting all other possible thermal reactions. Furthermore, thermal inertia and regulatory mechanisms in general limit the range of temperatures, and rates of temperature change that are achievable *in vivo*. In contrast, use of photons for activation energy to drive a given reaction allows comparatively exquisite control, because only certain molecules (chromophores) are excited, the excitation is to an excited state from which one or more specific chemical reactions may occur and finally, by varying wavelength and exposure time, the reactions can be readily controlled without "inertia." Use of ionizing radiation is less specific than use of optical radiation, because a multiplicity of ionization events always occur, and it is impossible to favor a given, exogenously-supplied chemical pathway to any great extent.

3. This radiation is transmitted into skin and cutaneous blood vessels and has an internal dosimetric depth profile; i.e. the penetration of 320–800 nm radiation into soft tissue is very wavelength-dependent because of the optical properties of the tissue.

In skin, the major epidermal chromophore in this wavelength region is melanin, which absorbs shorter wavelengths much more strongly than longer wavelengths, but has a continuous absorption spectrum across this entire spectral region. Although optical scattering does occur in the epidermis, it is absorption by melanin that essentially determines the transmission of incident radiation into and through the epidermis over the 320–800 nm region. In the dermis, however, optical scattering plays a major role in determining the depths to which longwave ultraviolet, visible, and near infrared radiation penetrates. Dermal optical scattering, primarily by collagen fibers, is much greater at shorter wavelengths, such that the relative depth of penetration of blue visible light into dermis is less than one-tenth that for red light. In addition, certain often sharp wavelength bands from 400 to 600 nm are strongly absorbed by the blood-borne chromophores, hemoglobin and oxyhemoglobin, and by bilirubin, which is both intravascular and extravascular. The precise fate of incident radiation within a person's skin depends on many individual variables for each layer, but it is clear from general models for the optics of skin that longer optical wavelengths penetrate deeper into the tissue. For example, whereas 320 nm radiation is largely attenuated in the first 100 μm of tissue, up to 20% of incident 800 nm radiation may be transmitted through the entire epidermis, dermis, and subcutaneous adipose layers. In particular, an "optical window" exists over the 600–1300 nm region, owing mainly to lack of strong absorption by blood and secondarily to lower optical scattering within the dermis. Amazingly, on the order of 1% of 700–800 nm radiation can penetrate the entire human chest wall, but less than 0.001% of wavelengths lower than 500 nm is transmitted (JA Parrish: Unpublished data).

A highly useful advantage of these optical properties is that the wavelength chosen for initiating a particular biologic response, release of drug, inactivation of enzyme, etc., will largely determine which skin layers are affected. With exogenously supplied chromophores having broad action spectra, the depth of tissue involvement might be controlled over a wide range. The activation or release of blood-borne drugs from a photolabile "package" might best be accomplished with wavelengths longer than 600 nm, or with shorter wavelengths chosen between the absorption bands of the hemoglobins. Under other circumstances, it may be advantageous to use 400–420 nm radiation because the very strong Soret absorption band of oxyhemoglobin could act as an "intravascular sunscreen" to protect circulating leukocytes, but allow interstitial cells to be affected by a given photochemical reaction sequence.

4. This radiation is absorbed by a variety of molecules and biomolecules which are nontoxic and can therefore be administered systemically.

The chemical structure of molecules will determine the specific wavelength of nonionizing electromagnetic radiation that will be absorbed. While fully saturated organic compounds generally absorb wavelengths below 200 nm and unsaturated bonds have absorption bands between 200 and 300 nm, conjugated double-bonds result in absorption bands between 200 and 400 nm. For larger conjugated molecules, and certain substituted aromatic compounds, absorption shifts further to longer

wavelengths; these compounds appear colored because of absorption of various visible wavebands. Although molar extinction at an absorption spectrum maximum can be proportional to the number of conjugated bonds, in biologic macromolecules such as proteins or nucleic acids, the absorption maximum is not a simple linear sum of the number of conjugated doublebonds. Absorption characteristics are affected by the addition of auxiliary charged side groups and in solution or *in vivo* absorption by compounds containing ionizable groups is pH dependent.

To function as a photosensitizer a molecule must usually be capable of more than merely absorbing a photon. In general it must be able to be excited by EMR into a long-lived excited state, the triplet state or react to form more long-lived chemically active species such as free radicals. With certain important exceptions, most of the photosensitized reactions in biologic systems studied to date involve the participation of molecular oxygen. Free dyes are efficient photosensitizers for many biomolecules including amino acids, proteins, and nucleic acids. Binding of the dye to the substrate, which often precedes photooxidation, changes the photosensitizer's efficiency. Acridines, thiazines, xanthenes, anthraquinones, azines, flavin and nonmetal containing porphyrins are often efficient photosensitizers of protein degradation. Methylene blue, flavin, psoralens and acridines are effective photosensitizers of nucleic acid reactions. Unsaturated fatty acid reactions are photosensitized by a variety of chemicals including certain porphyrins.

Clinical photosensitization provides evidence that systemically administered light absorbing chemicals can be activated *in vivo*. Hundreds of photosensitizing chemicals of therapeutic, industrial, agricultural, or other origin may reach viable skin cells via the bloodstream, each having its own pattern of absorption, metabolism, and binding to skin components. In general, these compounds are highly resonant structures with a molecular weight of less than 500 and absorb radiation in the ultraviolet and visible range. Some of the more common compounds include tetracyclines, especially demethylchlortetracycline, sulfonamides, especially sulfanilamide, griseofulvin, phenothiazines, especially chlorpromazine, thiazides, psoralens, sulfonylureas, and many others. Many are triheterocyclic compounds and most are fluorescent although fluorescence as such is not often correlated with photosensitizing effectiveness.

PHOTON DEPENDENT DRUG DELIVERY SYSTEMS

A potentially useful treatment system is a photon-activated drug delivery system (PADDS). The most direct PADDS would use photons to modify drugs *in vivo* in the same ways enzymes can be modified. Photochemically directed synthetic chemistry and the expertise and techniques developed for photoaffinity labeling could be utilized to design a variety of drugs for local activation or deactivation in specific tissue sites and depths after systemic administration. Binding, release, biotransformation and inhibition could be influenced by chromophoric prosthetic groups which could be photoisomerized, cleaved, or removed by specific wavebands of 320 to 800 nm radiation. Here lies an exciting challenge to the photobiochemist.

Another example of PADDS is the use of photolabile liposomes to deliver medications. Liposomes are ordered concentric closed membrane vesicles composed of phospholipid bilayers in aqueous suspension and enclosing an aqueous phase [20]. Because they are useful models for biological membranes, liposomes have been extensively studied [21–24] and their potential as drug carriers [25,26] has been recognized and tissue targeting has been examined. A variety of drugs can be trapped inside of liposomes whose properties of "leakiness," tissue distribution, stability and half-life can be markedly influenced by their lipid content and technique of preparation. For example, the addition of cholesterol results in decrease "leakiness" and sphinogmyelin confers resistance to the recognized disruptive effects of serum [27]. Cationic lipids or anionic polar lipids increase the volume of the aqueous phase within the liposome. A variety of preparation maneuvers result in small unilamellar vesicles (20–50 mm), large unilamellar vesicles (up to 100 nm diameter) [28] or multilamellar vesicles. Extrusion through polycarbonate membranes can be used to sterilize and standardize the size of the vesicles. Half-times for *in vivo* biodegradation or destruction of vesicles can be varied from 12 to 600 hours depending upon their composition [29].

Natural targeting to the reticuloendothelial system occurs but to some extent one can target liposomes by varying size and charge and route of administration [22] to increase distribution to specific organs such as liver, spleen or lung. Liposomes have been targeted to lung, nodes, liver, and spleen after intravenous administration, to nodes and skin after subcutaneous injection and have also been given intraarticularly [30] and intratracheally [31]. Liposomes have also been targeted to specific tissue sites by covalent binding of liposomes to antibodies to specific tissue antigens [32].

Heat has been used to cause *in vivo* release of drug from systemically administered liposomes [33]. Mixtures of dipalymitoyl and disteroyl phosophtidylcholine have a transition temperature slightly above body temperature, and at temperatures above transition temperature the liposomes rapidly become leaky. Elevated methotrexate levels were achieved in murine skin tumors heated to 42°C after intravenous injection of the drug entrapped in liposomes. The heat-induced disruption of liposomes intravascularly resulted in local accumulation of methotrexate in the tissue. Temperature dependent liposome drug delivery has some inherent restriction of lipid composition because addition of those lipids known to stabilize liposomes in serum may also alter the transition temperature.

Pure lipid liposomes can be disrupted by photosensitizers and EMR of appropriate wavelength. Toluidine blue and visible light of wavelengths longer than 520 nm causes lysis of liposomes as measured by glucose leakage or a change in light scattering. Before rupture occurs the membrane lipids undergo peroxidative damage which can be prevented by carotenoids [34,35]. Chlorpromazine and long-wave ultraviolet radiation causes oxygen dependent damage to liposomes and release of trapped spin label marker [36]. Exposures of retinal enriched liposomes to 365 nm radiation induces sensitized oxidation of the lipid bilayer and increased fluidity detected by spin label technique. This in turn leads to lysis of the liposome and release of entrapped chromate ions [37].

By adding photosensitized release mechanisms to the known techniques for liposome targeting it may be possible to selectively treat diseases of skin or localized tissue abnormalities. There are several photosensitizers known to cause membrane damage including xanthene dyes, thiazine dyes, porphyrins, chlorpromazine and others, which could be used to photosensitize liposomes to a specific wavelength of EMR which could then be used to alter the liposome membrane and release drugs from the liposomes *in vivo*. This would add to the ability to target drugs to specific anatomic sites (exposed sites) and tissue depths (dependent on wavelengths). Certain medications could be protected from biotransformation until released locally by EMR or rate of drug release could be increased or decreased by irradiation. Drug-bearing, light-sensitive liposomes could be a depot from which light could trigger periodic partial release to obtain intermittent or partial drug action or total release to terminate the action. A second photosensitizer could be released by the action of the first and subsequently utilized for photochemotherapy by radiation with a second waveband (PADDS-photochemotherapy).

Photosensitizers could be bound to the liposomes, incorporated into the membrane or entrapped within medicine-containing liposomes. The bilayer membrane would transmit the radiation absorbed by most photosensitizers. Membrane fusion could be used to join photosensitized-containing liposomes to drug-containing liposomes or to join liposomes to cell walls. Lipophilic photoactivatable reagents are used to identify lipid-embedded domains in membranes [38]. Compounds that first

dissolve in lipid bilayers and can subsequently be photoactivated have been developed [39–43]. Fatty acids containing photoactivatable groups have been synthesized [44–46] and these could be incorporated into the photolipids used to produce liposomes. This synthetic chemistry and photochemistry expertise may be utilized to produce nontoxic, light sensitive liposomes. Another challenge for the chemists.

MANIPULATION OF EXPOSURE VARIABLES

The precision, efficacy, and safety of all forms of therapeutic photomedicine can be enhanced by a variety of manipulations of the exposure source. The spectral power distribution can be selected to match the action spectrum of desired therapeutic results, to obtain verified waveband interactions, and to minimize side effects. Intensity can also be varied to give a designated dose over different periods of time. Shorter exposures may be more convenient, longer exposure may permit exposure of more blood flowing through the skin.

If one tissue response is related only to total dose and a second response is related to intensity as well, it may be possible to select one of the responses by varying the intensity while achieving the same total dose. For instance, the presence and degree of the delayed erythema component of ultraviolet-induced inflammation are dependent on exposure dose. For a given area, the exposure dose equals the produce of irradiance and exposure time. Within extremely wide limits ($>10^{10}$-fold range) [47] delayed erythema response is independent of irradiance ("dose rate"); that is, the influence of doubling the irradiance may be compensated for by halving of the exposure time (48). However, not all photochemically initiated biologic responses necessarily follow this "reciprocity law." For example, reciprocity over a wide range of irradiances is not the case for thermally induced photobiologic responses. As a site is exposed, heat transfer away from the exposure area occurs until at some equilibrium, the temperature attained at the exposure site and certain related responses such as immediate vasodilitation are proportional to the irradiance, and not the exposure dose. It may, therefore, be possible to select for certain competing photobiological or thermal responses in the host by varying intensity. Lasers may be very useful in this regard.

Additional advantages can be gained by altering not only the exposure conditions but also by altering the host. The sensitivity of skin to specific EMR wavebands can be decreased or increased by the topical application of a variety of chemicals. If absorbing or scattering compounds are applied to the skin, sensitivity to radiation in the spectral regions involved will decrease. On the other hand, aqueous media applied to normal Caucasian skin results in the extraction of UV-absorbing compounds from the skin, causing a corresponding increase in transmittance of the stratum corneum as appropriately measured *in vitro*, and an increase in sensitivity to ultraviolet radiation *in vivo* [49,50].

Other alterations of optical properties of skin are possible. Normal skin has a single, continuous though somewhat irregular air-tissue interface in which the refractive index (n_D) changes from that of air, 1.0, to that of stratum corneum (1.55) [51]. This causes a regular reflectance of 5% to 7% across the UV, visible, and near-infrared spectra [52]. Adding a layer of clear, lipophilic liquid such as mineral oil, which readily spreads over the surface of skin, and has a refractive index ($n_D = 1.48$) between that of air and stratum corneum, does little to reduce regular reflectance occurring at the optical interface with air. However, in abnormal tissue such as psoriasis, there are multiple air-tissue interfaces along the path of incident radiation as it enters the stratum corneum and regular reflectance occurs at each such interface. As a result the total regular reflectance is considerably greater than for the single air-tissue interface seen in normal skin. When mineral oil is applied to the scaly plaques typical of psoriasis vulgaris, it apparently fills air spaces between superficial flakes of corneocytes. Regular reflectance of the plaque is thereby decreased because the oil provides a much better match of refractive index *between* the flakes of corneocytes than does air [53]. Reducing regular reflectance in this manner must necessarily increase the fraction of incident radiation transmitted into the abnormal tissue. This is one example of selective increase in EMR transmission.

It is also possible to change skin temperature by applying conductive or radiative heat. This would alter the percutaneous and tissue clearance of certain photosensitizers or medications and also influence cutaneous blood flow. Blood flow can also be increased locally by pharmacological agents, by suction or by prior exposure to more erythemogenic shorter wavelength ultraviolet radiation (250–300 nm). Vessel permeability can be altered pharmacologically to release more photosensitizers or medication into tissue. Selective protectors or quenchers could also be given systemically or locally released in order to spare the host from certain known side effects. All of these changes may be combined to deliver more drug bearing liposomes, blood-borne photosensitizers, or medications to the heated tissues or to skin.

The manipulation of any one of these exposure dose parameters may have only small influence on treatment efficacy or side effects but their combined effect could make therapeutic photomedicine a safer and more useful modality and a challenging science.

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